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AUDITORY EVOKED POTENTIALS FROM THE FROG EIGHTH NERVE

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AUDITORY EVOKED POTENTIALS FROM THE FROG EIGHTH NERVE

Summary: A method for recording evoked potentials from the eighth nerve of frogs using midline and lateral electrodes is described. A prominent bipolar wave recorded at 3-6 ms is attributable to eighth-nerve activity. The evoked potential provides an integrated response for study of inner ear activity.

Keywords auditory, evoked potential, eighth nerve, frog

The auditory system of the frog has previously been studied with evoked potentials (among other techniques) at several levels of the nervous system^{3,10,12-14,16-17}. Although electrodes were placed into neural tissue in most of the studies, in one case wires placed through holes in the skull midline were used³. The midline evoked potentials had multiple peaks which were attributed to unspecified centers of neural activity³. In the present study, evoked potentials were recorded using a midline electrode and an electrode placed lateral to the inner ear to enhance the recording of the eighth-nerve component.

Bullfrogs (*Rana catesbeiana*, Parc-Bio, Wenonah, NJ) 94-138 g of either sex were used in experiments. Anesthesia was initially induced with ketamine (75 mg/kg) sodium and pentobarbital (37.5 mg/kg) injected into large muscles of opposite hindlimbs and was subsequently maintained with ketamine. After absence of reaction to hindlimb pinches, the anesthetized frog was placed dorsal side up on a foam rubber pad in an electrically shielded room. Experiments were conducted at room temperature (18-23°) with the room darkened. The background noise level was 47-50 dBA.

Two sites were prepared for recording electrode. The midline site was prepared by first making a midline incision approximately 3 cm long in the skin over the dorsal skull. After minor reflection of muscle using blunt dissection, a 1-mm hole was drilled in the dorsocaudal aspect of the frontoparietal bone. A lateral site was prepared by making a 1-2 mm incision in the skin immediately posterior to the left tympanum and wall of the middle ear.

Transient acoustic stimuli were delivered by Etymotic ER-3A earphones connected in parallel to a Wavetek 164 sweep generator. The end of each flexible earphone tube was placed inside a rigid plastic tube with the terminal foam eartip (ER-3-14) forming an acoustic seal. The rigid tube provided support and directed the acoustic energy. For airborne stimuli, the 8-mm diameter opening of a support tube was positioned approximately parallel to and 1 mm

away from a tympanum. Sound measurements were made using a calibrated Brüel and Kjaer system with a 1/2-inch Larson-Davis 2541 microphone placed in the tympanum location. A single-cycle 1-kHz sinusoid signal applied to the earphones resulted in the acoustic waveform shown pressure level (dB SPL re 20 μ Pa) of continuous tones produced by the same amplitude sine wave. Acoustic stimuli of 50-100 dB were delivered to ears bilaterally (BA, binaural), to the ipsilateral left ear (IA, monaural), or to the contralateral right ear (CA, monaural).

Electrodes were fashioned from common copper wire or Polypenco carbon-loaded Teflon (Polymer Corp.) by cutting a conductor and its insulation at right angles to the conductor axis. The Polypenco insulation was trimmed back 0.5-1 mm in some cases to provide an extended conductor. One electrode was inserted into the skull midline hole with its end approximately flush with the inside of the skull. The other electrode was inserted through the lateral skin incision and along the middle-ear wall for 0.5-1 cm. The midline and lateral electrodes were connected to the noninverting and inverting inputs, respectively, of a Grass P16 preamplifier (rise time, 100 μ s; time constant, 30 ms; 4 Hz - 3 kHz, half amplitude points). A third electrode with about 2 cm of exposed conductor was inserted under dorsal skin through a small skin incision 1 cm anterior to the vent and connected to the preamplifier ground. Further amplification by a Grass P18 preamplifier (rise time, 30 μ s; DC - 11 kHz) gave a total gain of 1000.

The amplifier output was fed to a Wavetek 804A signal processor. For each stimulus condition, 200 responses to stimuli delivered every 1.2-1.4 s were averaged using a bandwidth of 5 kHz. Each averaged response was plotted using a Hewlett Packard (HP) 9872C digital plotter and was stored on disk. The signal processor and the sweep generator were triggered simultaneously with a free running HP 8011A pulse generator.

A distinctive evoked response lasting 8-12 ms was recorded. Potentials with longer

latencies were observed but not studied. Examples of recorded waveforms evoked by bilateral airborne stimuli are shown in Fig. 2. A response began with a negative-positive wave (peaks N_1 and P_1) for all stimuli. One or two negative peaks (N_2 and N_3) occurred in the subsequent 7 ms with latencies and relative amplitudes variable among animals and stimulus conditions. This shape was observed for all stimuli used; however, relative amplitudes of the later negative peaks, as well as the overall response amplitude, were sensitive to location of the midline electrode.

Latencies and amplitudes of the evoked potentials of Fig. 2 are plotted as the BA data in Figs. 3 and 4. The same measures are also plotted for airborne IA and CA responses, which had shapes similar to BA responses. The latencies do not include the 1-ms delay between electrical and acoustic signals. The two figures illustrate the latency decrease and amplitude increase seen for increasing intensity of each type of stimulus. They also show the longer latencies and smaller amplitude of the IA and CA responses relative to the BA response. Similar differences were seen in all experiments.

Evoked potentials were also recorded with the opening of one earphone support tube held against the ipsilateral forelimb and the contralateral body wall. The intensity of these intended vibratory stimuli was not measured. The second tube was removed from the vicinity of the frog and covered with gauze. In each case, the response consisted of an initial negative peak, a positive peak, and a later ill-defined negative wave. Latencies of the first two peaks were comparable to those of initial peaks in responses to 60-70 dB airborne stimuli: 4.0 and 5.2 ms for the ipsilateral leg, 4.5 and 5.7 ms for the contralateral body wall. The amplitude was less than 2 μV . The positive peak and subsequent negative peak were broader than the corresponding peaks in responses to airborne stimuli.

Previously reported response latencies of single-unit action potential occurrences and

evoked-response peaks in *Rana* to airborne stimuli are 3-7 ms in eighth nerve⁵, 6-50 ms in dorsal medullary nucleus^{1,6,7}, 10-50 ms in superior olivary nucleus⁶, 10-100 ms in torus semicircularis^{1,2,4,14,16-18}, 30-120 ms in thalamus^{7,11,13,14}, and greater than 30 ms in telencephalon¹². The short latencies in the ranges of 2.5 to 4 ms for N_1 and 3.5 to 5 ms for P_1 (Fig. 3) and the larger relative N_1 - P_1 amplitude (Fig. 2) recorded with electrodes along the eighth nerve are evidence for the eighth-nerve origin of the initial bipolar wave of the response. Based on the additional latencies of 3-5 ms (BA and IA) and the above latency data, the negative peaks N_2 and N_3 at 5-12 ms are most likely generated in dorsal medullary and superior olivary nuclei. The additional delays are sufficient for action potential propagation to and synaptic delay in the ipsilateral dorsal medullary nucleus, and for subsequent propagation to the contralateral dorsal medullary nucleus and/or ipsilateral and contralateral superior olivary nuclei¹⁹.

The responses demonstrate that evoked potentials can be easily recorded from the eighth nerve in the frog using implanted electrodes. In one animal an evoked potential was recorded with electrodes inserted short distances into small skin incisions over midline and lateral electrode sites. The response (1.6 μ V for 78.5 dB BA stimulus) was smaller but similar in shape and time course to responses recorded with implanted electrodes. Thus, a surface eighth-nerve evoked potential is also feasible but may require additional processing.

The evoked potentials recorded with the implanted electrodes were generally similar to those previously reported for click stimuli and electrodes at midline sites³; both responses had similar number of peaks occurring within 15 ms of stimulus onset. The initial bipolar wave recorded using the midline-lateral configuration was consistently and substantially larger than subsequent peaks (Fig. 2). On the other hand, responses recorded in preliminary experiments with both electrodes at midline sites were smaller and were dominated by positive waves at 5-8 ms and a subsequent negative slow wave. An initial negative-positive wave was not always

evident in the midline recordings. Although initial negative peaks in the previous study had latencies not much different than the ones reported here, their amplitudes were similar to those of later peaks in many of the responses³.

Since the frog can sense extratympanic body and substrate vibrations^{8,9,15,20}, the eighth-nerve evoked potential described here is most likely a composite auditory and seismic sensory response. Due to the nature of the electrodes, integrated inner ear responses were recorded. Responses attributable to the separate sensory areas of the inner ear are not evident in the responses as recorded and processed, but they may be responsible for inflections seen near the middle of the initial bipolar wave in some recordings. More detailed analysis of the first 5-6 ms of responses will be required to address the possibility of separating individual sensory responses. It may also be necessary to position electrodes more precisely to achieve greater spatial resolution in the recorded volume.

Amplitude of the evoked response was repeatable over several hours for a given electrode position in an animal, allowing time for several different stimuli to be presented. Respective latencies were consistent for repeated presentations of a given stimulus. Regardless of the future ability to record responses to stimulation of specific sensory epithelia preferentially, the method can be used for assessment of relative effectiveness of various stimuli in the inner ear. A response of this type provide an integrated measure of inner ear activity which complements used stimulus strategies such as variation of frequency and addition of masking stimuli.

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FIGURE LEGENDS

Fig 1. The airborne acoustic stimulus waveform. A single-cycle 1-KHz signal drove the earphone. The rms equivalent is 78.5 dB SPL.

Fig. 2. Potentials evoked by bilateral airborne stimuli in two animals. The number in each panel is the peak intensity of the acoustic stimulus in dB SPL. Vertical lines mark the

application of the electrical signal to the earphones, with each recording starting 5 ms before the application. There was a delay of 1 ms between the triggered signal application and arrival of the acoustic stimulus at the tympana. Each response is displayed for 35 ms after the stimulus trigger. The vertical line in the bottom panel represents 20 μ V for frog 3 and 10 μ V for frog 2.

Fig. 3. Response latency. Data are for bilateral (BA), ipsilateral (IA), and contralateral (CA) stimuli. Open symbols represent data from frog 2; filled symbols, from frog 3. Diamonds represent latencies of a single late negative peak. From bottom to top, the data are for N_1 , N_1 , N_2 , and N_3 for each type of stimulus.

Fig. 4. Amplitude of the N_1 - P_1 bipolar wave. Data are for bilateral (BA), ipsilateral (IA), and contralateral (CA) stimuli. Open symbols represent data from frog 2; filled symbols, from frog 3.

Fig. 1

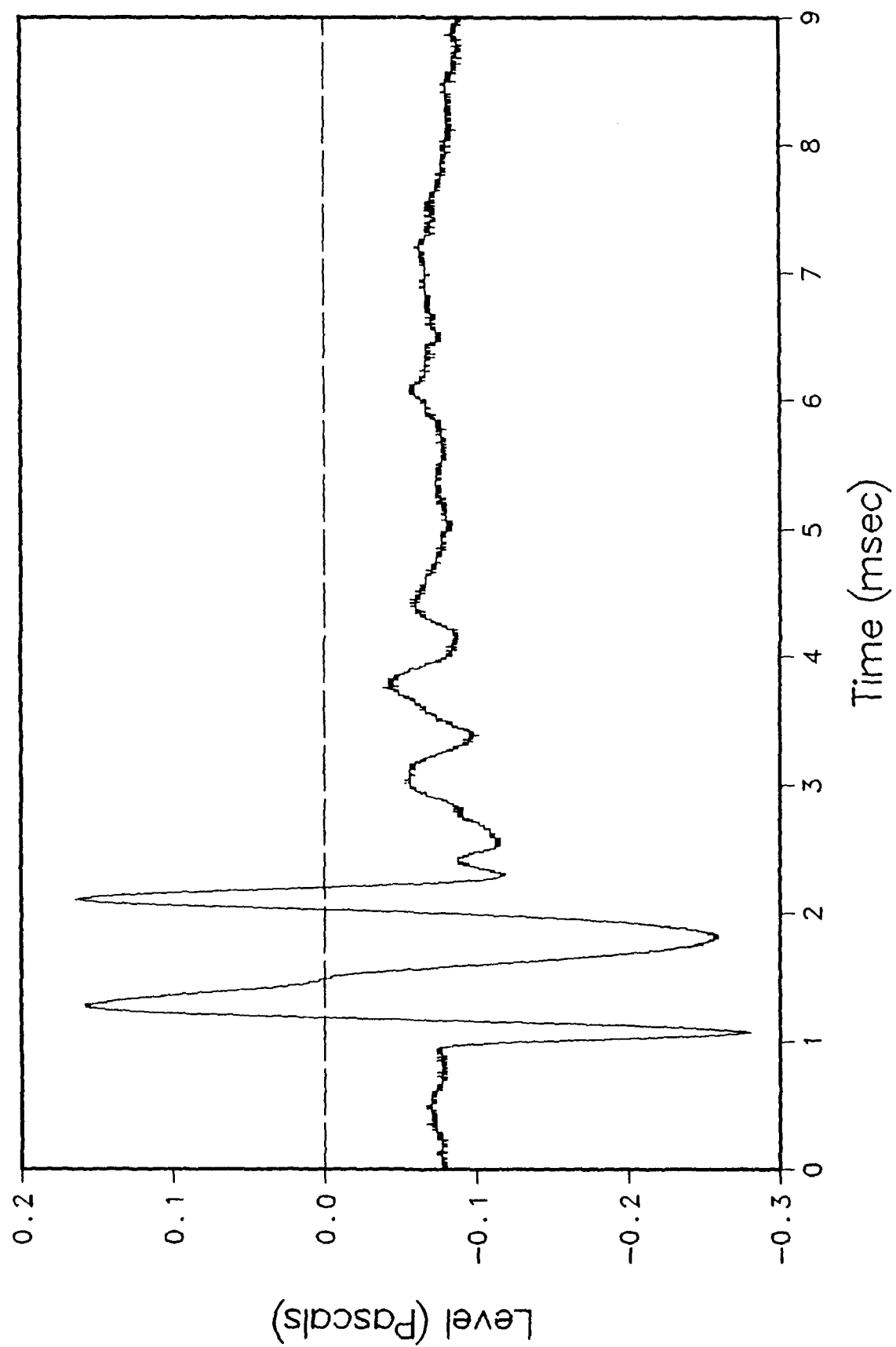


Fig. 1

Fig. 2

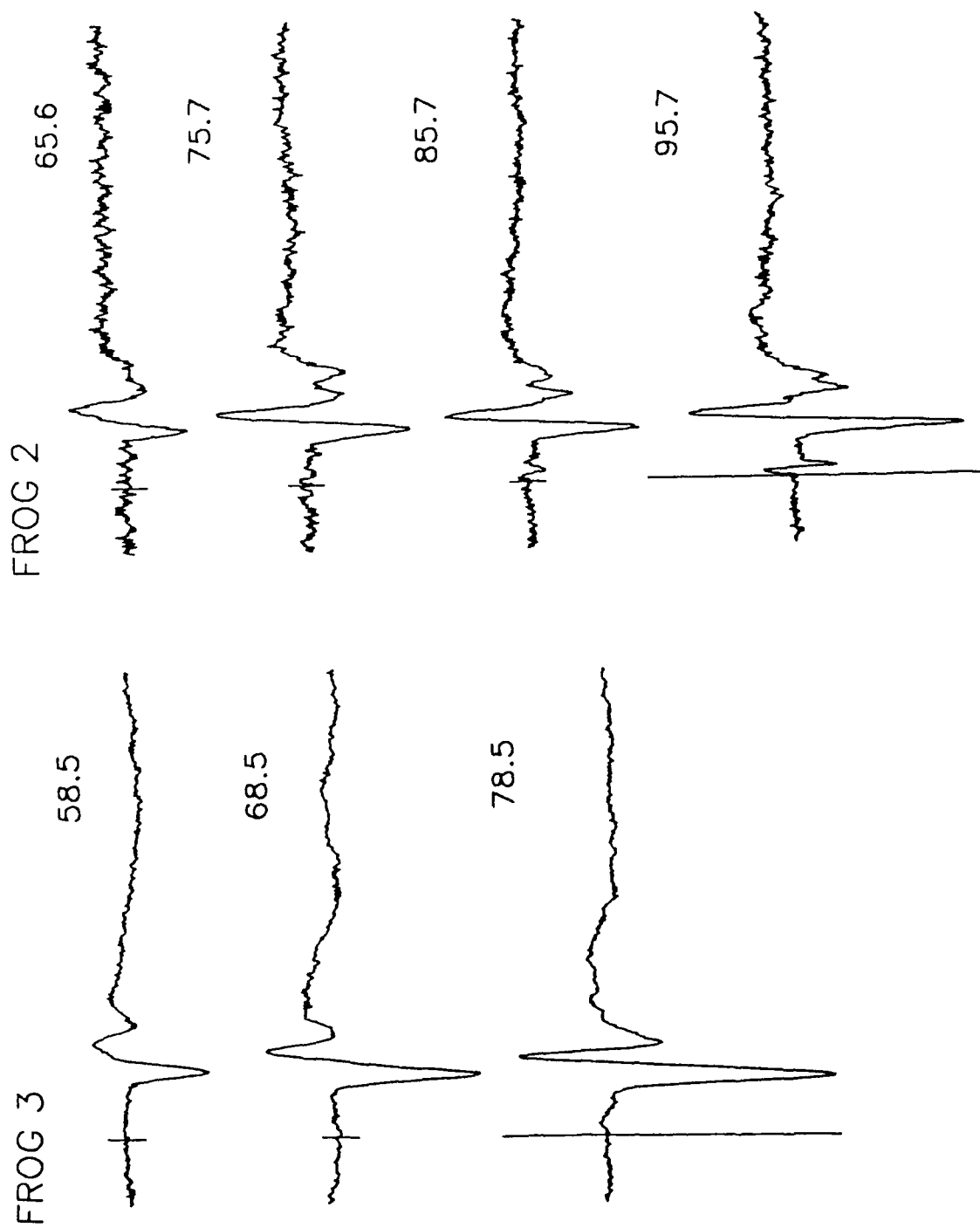


Fig. 2

Fig-3

Fig. 3

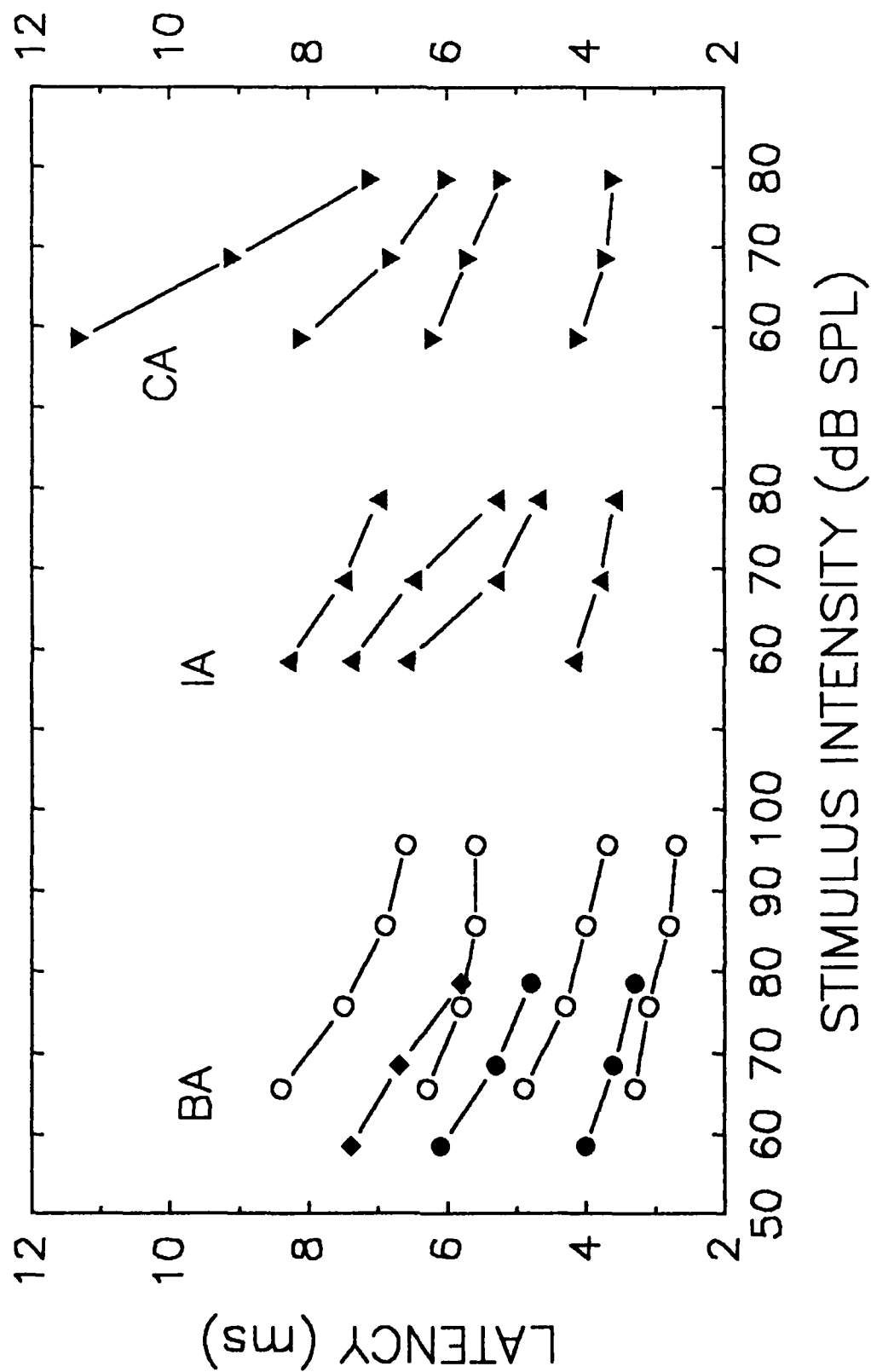


Fig. 4

Fig. 4

